REPORT TITLE

Endosulfan Task Force Response to the Health Effects Division
FQPA Safety Committee Report
Dated February 14, 2002:

Endosulfan – Report of the FQPA Safety Factor Committee

DATA REQUIREMENT
Not Applicable

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

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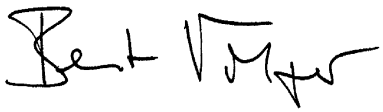
No Good Laboratory Practice Statement is required for the information presented in this volume according to 40CFR Part 160.



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Date: April 5, 2002

ENDOSULFAN TASK FORCE RESPONSE TO THE HED's "ENDOSULFAN – Report of the FQPA Safety Factor Committee"

RE: Response to Comments on EPA's memorandum ENDOSULFAN – Report of the FQPA Safety Factor Committee 02/14/02, PC Code: 079401, (*Correspondence: D. Locke and E. Mendez to C. Christensen February 7, 2002*).

INTRODUCTION:

The subject document was received by the Endosulfan Task Force (ETF) on March 15, 2002. When the ETF first learned about this report, we were very surprised and disappointed about the timing and nature of this assessment considering the actual stage of the endosulfan reregistration process (comment period closed officially on November 13, 2001). The subject Committee decided to raise the FQPA safety Factor to 10x. As indicated in our Phase 3 responses, the ETF already considered the originally assigned FQPA Safety Factor of 3x (10/20/98) as excessive and not justifiable, since the existing database clearly shows no evidence of increased susceptibility to young animals. Raising the Safety Factor to 10x is even less comprehensible and justifiable as explained below in detail.

Despite the inappropriate timing within the reregistration process, we still welcome the opportunity to respond on the latest hazard assessment made by the FQPA Safety Factor Committee, and would appreciate that these comments will be taken into consideration by the Agency for further review in their future assessments. Our comments focus on Section I - Hazard Assessment, part 2 – *Determination of Susceptibility,* part 4 – *Evidence for Endocrine Disruption,"* and Section III – Safety Factor Recommendation and Rationale.

EPA COMMENTS:

In the various draft phases of the HED Toxicology Chapter for the Endosulfan RED, the Agency provided several Hazard Identification Assessment Review Committee (HIARC) reports (10-7-98 HED DOC. 012901; 01-31-00 TXR No. 014024; 02-28-02 TXR No. 0050518). In each of these reports the HIARC concluded, "*The database for endosulfan is complete and there are no data gaps pertaining to developmental or reproductive toxicity. The data provided no indication of increased sensitivity of rats or rabbits to in utero and post-natal exposure to endosulfan. Two prenatal developmental toxicity studies, one in rats and one in rabbits failed to show evidence of developmental toxicity in the absence of maternal toxicity. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity."*[1] These reviews also included evaluation of and comments regarding available public literature. The conclusions of the HIARC, which were forwarded to the FQPA Safety Factor Committee, provided acute, short-

[1] **Fricke, R.F. *ENDOSULFAN: RE-EVALUATION of Toxicology Endpoint Selection for Dermal and Inhalation Risk Assessments and 3X Safety for Bioaccumulation* – Report of the Hazard Identification Assessment Review Committee. TXR No. 0050518. February 28, 2002 PC Code: 079401**

term/intermediate and chronic toxicity endpoints for appropriate dietary and occupational risk assessments. The HIARC also identified data gaps that included a subchronic neurotoxicity study – rat and a developmental neurotoxicity study – rat (held in reserve).

The FQPA Safety Factor Committee's initial assessment in November 1998 concluded:

> *"The FQPA Safety Factor Committee concluded that the FQPA safety factor is required, however can be reduced to 3x because: 1) there is no evidence of increased susceptibility in any study; 2) the severity of the fetal effects in the reproduction study were not consistent between generations and the target organ toxicity seen in this study was not seen in any other study; and 3) reliable data and conservative assumptions in screening level models were used to assess the potential dietary (food and water) and residential exposure to this chemical. Consequently the FQPA safety factor was reduced based on the uncertainty associated with the data gap for a developmental neurotoxicity study in rats*."

However, following the HIARC meeting in February 2002, the FQPA Safety Factor Committee revised their assessment and provided the following recommendation and rationale:

> *"The Committee concluded that the 10x FQPA Safety Factor should be retained. Previously (November 20, 1998), the Committee recommended a 3x FQPA Safety Factor due to the lack of a DNT. At the current meeting, however, the Committee recommended that the 10x FQPA Safety Factor should be retained because there was not reliable data available to address the following concerns or uncertainties raised by the following matters: 1) evidence for increased susceptibility of the young rats, 2) additional evidence for endocrine disruption, 3) uncertainty regarding the neuroendocrine effects in the young, and 4) the need for a DNT."*

The ETF believes that this reassessment is inappropriate and scientifically unjustified. This report from the ETF specifically addresses the four issues identified above, which were in the February 2002 FQPA Safety Factor Committee report. In general, the ETF believes that substantive data does exist within current guideline and public literature studies that provide reliable information to address, to a great degree, the uncertainties of these matters, and a 10x FQPA safety factor is excessive and unwarranted.

ETF RESPONSE:

Page 2, Section I. Hazard Assessment: 2. *Determination of Susceptibility*

In this section the FQPA Safety Factor Committee provided the following comments regarding susceptibility:

> *"A recent review by the Agency for Toxic Substances and Disease Registry (ATSDR) reported the results of non-guideline studies which demonstrated that young rats may be more susceptible than older rats upon exposure to endosulfan. Studies conducted by Zaidi et al. (1985) and Sinha et al. (1995 & 1997) illustrate*

effects to the offspring at doses lower than those showing effects in adults. In the first, neonatal rat pups were dosed for 25 days intraperitoneally and displayed increased serotonin binding to the frontal cortical membranes of the brain and increased aggressive behavior. Adults exposed in a similar manner did not display these effects. In a study by Sinha et al., both three week and three months old rats were treated orally; decreased intratesticular spermatid count and increased percentage of abnormal sperm were seen in three week old rats at doses lower than those eliciting similar effects in three month old rats."

The ETF has reviewed the ATSDR document, as well as the referenced papers and concurs with the ATSDR's conclusion, "*No reports of adverse effects in endosulfan-exposed children were found, but it is reasonable to assume that children will exhibit similar signs and symptoms to those in adults under similar exposure conditions. Some studies in animals have provided evidence that young animals respond to endosulfan differently than adult animals (Kiran and Varma 1988; Lakshmana and Raju 1994; Sinha et. al. 1995, 1997; Zaidi et. al. 1985), but there is no conclusive evidence to suggest that young animals are more susceptible than older ones."*[2]

In response to the first citation (Zaidi *et al.,* 1985), the ETF conducted a literature search on endosulfan and neurobehavioral effects. The ETF located nine citations generated from several different co-investigators from three laboratories in India. Co-investigators from the Industrial Toxicology Research Center included Zaidi, Seth, Anand and Agrawl. A series of studies looking at serotonin binding in the brain and behavioral responses (foot-shock induced fighting response and conditioned avoidance using foot shock and pole climbing escape) were conducted by this laboratory in the mid-1980's (1983-86). Zaidi (1984) dosed neonates from PND 1-25 or PND 1-35 intraperitoneally with endosulfan at 0.5 and 1.0 mg/kg/day. Evaluations at post-natal day 25 did not show significant changes in receptor binding or fight response. There was a slight increase ($p<0.05$) in frontal cortex binding of 5-HT (serotonin) and fight response at 1 mg/kg/day at post-natal day 35. In contrast, a study by Seth (1986) treated adult rats with 1 mg/kg/day for 30 days and showed no effect on binding of 5-HT or fighting response. However, papers by Anand (1985) and Agrawal (1983) did show increased binding and increased fighting responses in adult female (septal-lesioned) and male (normal) rats at a slightly higher dose of 3 mg/kg administered for 15 to 30 days. In addition, studies conducted by Paul (1993 and 1994), from the Post-Graduate Institute of Medical Education and Research in India also showed increased 5-HT binding in the cerebellum and midbrain in immature rats administered 2 mg/kg/day for 90 days via gavage. Lastly, Chugh (1994), a co-investigator with Paul, showed enhanced learning and memory in 6-week-old mice administered a single dose of endosulfan at 1 and 2 mg/kg 30 minutes just prior to or just after conditioned stimulus. The small sample sizes, differences in technical material, vehicle, rat strain and route of exposure, as well as limited representation of measured data in the publications and use of dose levels known to be systemically toxic, makes it nearly impossible for the ETF, as with ATSDR, to derive sound scientific conclusions concerning potential sensitivity to young animals vs. adult from these data sets.

[2] **ATSDR Toxicity Profile for Endosulfan. September 2000. P.166**

The ETF also conducted a literature search looking at data related to endosulfan exposure and potential spermatogenetic effects in rats. There were seven citations representing three authors/laboratories from India. Of these, work by Sinha *et al.* (1995, 1997 and 2001) and Dalsenter *et al.* (1999) specifically looked at spermatogenesis and sexual maturation in prepubertal and mature rats exposed to endosulfan during sexual differentiation (*in utero)* and maturation (lactation). The FQPA Safety Factor Committee report highlights effects on spermatid count and sperm morphology noted in two Sinha papers (1995 & 1997). In addition to these two studies, there are two new studies, which also looked at these parameters (Sinha et al., 2001; and Dalsenter et al., 1999). A weight-of-evidence evaluation of these studies, as well as available submitted guideline studies, does not support the Committee's concern regarding potential sensitivity to younger animals.

In the original study, Sinha et al. (1995), three-month-old Druckrey rats were dosed for 70 days at levels of 2.5, 5.0 and 10.0 mg/kg/day. The results of this study showed decreased sperm count in the cauda epididymis and daily sperm production, which were both related to a decrease in spermatid count. There was also an increase in abnormal sperm morphology at 5.0 and 10.0 mg/kg. While there was a non-statistically significant decrease in spermatid count at 2.5 mg/kg, the decrease in both daily sperm production and sperm count in the cauda epididymis, directly related spermatogenetic processes, were statistically different.
The results from the Sinha et al. (1995) are consistent with those noted in the second study (Sinha et al. 1997), in which immature Druckrey rats (PND 21) were dosed at the same levels of endosulfan for the same length of time. The timing of this dosing scenario correlates to the first full cycles of spermatogenesis occurring at puberty. A literature review by Ulbrich et al. (1995) has shown that evaluation of spermatological and hormonal endpoints prior to 90 days of age can produce highly variable results. This is primarily due to the fact, that in rats, the first several cycles of spermatogenesis that occurs at puberty are highly inefficient and result in high cell death at various points in the spermatogenetic cycle.[3] Therefore, the decrease of spermatid count seen in the animals who started dosing at PND 21 versus those starting dosing at PND 90 should not be considered a reliable indicator of sensitivity to endosulfan. Taking into account that spermatological endpoints are highly variable and not very predictive, the overall change in the three related parameters (spermatid count, daily sperm production and sperm count in the cauda epididymis) was not significant between the 3-week and 3-month old animals. In addition, analysis of the statistical significance in sperm morphology reported in two studies showed an error at the 2.5 mg/kg/day dose level in study with 3-week old animals (Sinha et al., 1997). A re-analysis using the Tukey test showed a non-statistical change at 2.5 mg/kg, contrary to what was originally reported. There again, the results of this parameter are consistent with the findings in the previous report (Sinha et al., 1995) and do not represent an indicator of increased sensitivity in young animals.

The most recent study by Sinha et al. (2001) exposed Druckrey rats *in utero* only and

[3] **Russell, L.D., Alger, L.E., and Nequin, L.G. (1987). Hormonal control of pubertal spermatogenesis. Endocrinol. 120, 1615-1632.**

then evaluated sperm parameters at postnatal day (PND) 100. In this study decreases in absolute and relative testis, seminal vesicle, and epididymis weights were noted. No change was noted in prostate or body weight. A decrease in spermatid count and sperm count in the cauda epididymis was seen at both dose levels. Again, while these results may appear to suggest potential spermatogenetic effects in young animals, extreme caution should be used in evaluating data from a single laboratory and in a strain of rat that is unique to that laboratory (internal colony). In addition, these results have not been reproduced in studies conducted in different laboratories with different strains of rat.

In a study by Dalsenter et al. (1999), Wistar rats were exposed to concentrations of endosulfan of 1.5 and 3.0 mg/kg/day from GD15 – PND21. The exposure period is important, since unlike the Sinha et al. 2001 study, animals were exposed not only during sexual differentiation, but also through sexual maturation of all of the significant primary and accessory sex organs (epididymis – peak differentiation by PND 25; prostate – development of lobes, lumen and secretory glands PND 1-21; and seminal vesicles – formation of lumen, secretory gland and expansion PND 2-24). Animals were then evaluated prepubertally (PND 65) and at maturity (PND 140). Administration of 3.0 mg/kg/day caused maternal toxicity with decreases in body weight, increased pup mortality, and decreased pup weight. There was no effect on testis decent or preputial separation. A statistically significant increase in testis weight was noted at both PND 65 and 140 at 3.0 mg/kg (this is in contrast to a decreased weight in the Sinha et al. 2001). There was no effect on any other sex organ weights. There was a slight decrease in sperm production at the high dose, but no effect on sperm count in the cauda epididymis or sperm morphology. There was also no significant effect on serum testosterone levels.

A significant deficiency in all of these studies is a lack of histopathological evaluation of the gonadal organs, as well as key homeostatic organs, such as the liver, kidney, adrenals and pituitary. Recent work by a variety of laboratories has been conducted to support the on-going validation of *in vivo* studies for the evaluation of endocrine-mediated effects. This work has shown that histopathological changes are the earliest and most reliable indicators of endocrine-mediated effects. These studies have also shown that spermatological endpoints are highly variable and sensitive to external factors such as stress and circadian fluctuations, and were not reliably predictive of endocrine-mediated effects. [4]

Lastly, the guideline two-generation study (MRID# 00148264, see Attachment B: Summary) not only covers all periods of sexual differentiation, puberty, and maturation, but it provides critical information on histopathological changes in primary and accessory sex organs, as well as critical homeostatic organs (pituitary, liver, kidney). In this study Sprague-Dawley rats were administered endosulfan at levels of 0.2, 1.2 and 6.2 mg/kg/day in diet from 10 weeks pre-mating through mating, gestation, and lactation. There were no effects seen in any generation on sexual differentiation, sexual maturation, or fertility. In addition, there were no histopathological effects noted in any gonadal

[4] **Fricke, R.F., *ENDOSULFAN: Re-evaluation of Toxicology Endpoint Selection for Dermal and Inhalation Risk Assessments and 3X Safety for Bioaccumulation* – Report of the Hazard Identification Assessment Review Committee. TXR No. 0050518. February 28, 2002 PC Code: 079401**

organs or on the pituitary. There were effects on the two main homeostatic organ systems, liver and kidney. This data is not only substantive and reliable, but directly relates to factors that are most pertinent to assessing human risk (sexual differentiation and maturation, fertility and neonatal effects).

Based on the data available and a science-based weight-of-evidence, the ETF concludes that there is no evidence of increased sensitivity to young animals exposed to endosulfan. This was also the conclusion of the ATSDR.

Page 3, Section I. Hazard Assessment: 4. *Evidence of Endocrine Disruption*

The ETF has submitted three responses concerning endocrine disruption and endosulfan (MRID# 44939102, dated October 4, 1999; MRID# 45300203, dated January 5, 2001; and MRID# 45619001, dated February 28, 2002). Many of the issues discussed by the FQPA Safety Factor Committee have been previously addressed in the aforementioned responses. The ETF request that the Agency review the second ETF response (MRID# 45300203) that included a detailed summary and evaluation of many of the newest public literature citations regarding hormonal interactions and *in vivo* reproductive organ effects, which the Agency refers to in their recent report of the FQPA Safety Factor Committee.

A. Evidence in Regulatory Guideline Submitted Studies

In the FQPA Safety Factor Committee report the Agency cites potential evidence of endocrine effects from submitted, as well as public literature data. Effects noted in submitted studies included testicular atrophy and parathyroid hyperplasia in chronic toxicity/carcinogenicity studies in rats, and increased pituitary and uterine weights in a two-generation reproductive toxicity study in rats.

The Agency has repeatedly noted effects from a 1978 NCI chronic oral toxicity study in rats (MRID# 00004256). This study was not guideline acceptable due to excessive toxicity at the low and high doses. Effects such as testicular atrophy and parathyroid hyperplasia were a direct result of frank systemic toxicity that was seen at both doses, with mortality rates of 38% and 50% that resulted in termination of dosing at 74 and 82 weeks, respectively. Male rats in both dose groups also showed significant liver toxicity and chronic renal failure. As stated in the ATSDR document, the parathyroid hyperplasia was considered to be secondary to chronic renal failure.[5] Severe intoxication, which involves organs such as the liver and kidney, results in significant disruption of physiological homeostasis and indirect effects on the major endocrine axes. More importantly, there is no indication of these types of effects occurring in guideline accepted chronic studies in rats where the MTD was met, but not exceeded (MRID# 41099502 & # 41229001).

[5] **ATSDR Toxicity Profile for Endosulfan. September 2000. P. 64**

The FQPA safety Factor Committee has also shown concern regarding uterine and pituitary weight changes seen in the two-generation rat reproductive toxicity study (MRID# 00148264). This concern is in direct contrast to the conclusions of all three HIARC reviews that concluded:

> *"The data provided no indication of increased sensitivity of rats or rabbits to in utero and post-natal exposure to endosulfan. Two prenatal developmental toxicity studies, one in rats and one in rabbits, failed to show evidence of developmental toxicity in the absence of maternal toxicity. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity."*[6]

Again, these issues were discussed in detail in the EFT January 2001 response (MRID# 45300203). The purpose of the multi-generation reproductive toxicity study in rats is to measure possible disturbances of reproductive performance, development and maturation including development of sex organs (vaginal opening, cryptorchidism, etc.) at doses up to and including parental toxicity. Endosulfan, administered to both male and female rats, did not cause any such interference through two successive generations (MRID# 00148264). There was an indication of weight effects on the pituitary gland of the F_0 pups of the first mating and uterus of the F_{1b} pups from the first mating. These effects are of limited significance since neither the pituitary or uterus was seen as a target organ in any other study, there were no supporting histopathological changes noted, nor were these effects consistent across generations. In addition, four separate uterotrophic assays were negative for uterine effects at doses up to 100 mg/kg bw/day, suggesting that the weight-of-evidence is negative for specific endocrine effects on the uterus.

Table 1: Uterotrophic Assays for Endosulfan

Type of *in vivo* study	Endpoints	Endocrine Effects
Competitive binding to rat uterus ER *ex vivo* (Wade et al. 1997)	Estradiol binding to rER	Endosulfan inhibits estradiol binding only at excess. The number of ER and PR in uterus was unchanged
Competitive binding to mouse uterus *ex vivo* (Shelby et al. 1996)	Estradiol binding to mER	No competitive inhibition at 10^3 fold excess
Uterotrophic assay in sexually immature Sprague-Dawley rats (3 mg/kg/day i.p. on day 18-20 of age) (Wade et al. 1997)	Uterus: growth, peroxidase activity, number of PR/ER; Pituitary: weight, hormones (GH, prolactin, TSH, LH, FSH); Serum: Thyroxin	No uterotrophic activity or hormonal changes. DES caused increase in uterus weight (80%), peroxidase, prolactin and a decrease in number of ER
Uterotrophic assay in sexually immature CD 1-mouse (10 mg/ kg bw/day s.c. on days 17 -19 of age) (Shelby et al. 1996)	Uterine growth	No increase in uterine wet mass. DES, E_2, (4-OH)-tamoxifen, DDT, methoxychlor were positive

[6] **Fricke, R.F. *ENDOSULFAN: RE-EVALUATION of Toxicology Endpoint Selection for Dermal and Inhalation Risk Assessments and 3X Safety for Bioaccumulation* – Report of the Hazard Identification Assessment Review Committee. TXR No. 0050518. February 28, 2002 PC Code: 079401 p.21**

Type of *in vivo* study	Endpoints	Endocrine Effects
Uterotrophic assay in sexually immature AP-Wistar rats (5 – 100 mg/kg bw/day s.c. for 3 days) (Ashby et al. 1997)	Uterine growth	No increase in uterine wet mass. Estradiol and methoxychlor were clearly positive.
Uterotrophic assay on young ovariectomized female Wistar rats (Raizada et al. 1991)	Uterus / cervix / vagina wet weight and glycogen content; pituitary weight; histology	No effects after gavage of 1.5 mg/kg bw/day for 30 days although transient clinical signs were present.

Lastly, the statistically significant increase in pituitary weights was due to a single female in the high dose group (see Attachment A). When the outlier is removed, there is no statistical difference between the treated group and the concurrent controls. Also, as was stated above, there were no histopathological changes seen in the pituitary gland and it has not been shown to be a target organ of endosulfan in any other toxicity tests.

B. Evidence from Published Literature

The FQPA Safety Committee cited the following:

> ***"****The ATSDR, 2000 reported a number of studies that assessed endosulfan's effects on the endocrine system. Singh and Pandey (1989) dosed adult rats orally for 7 days and observed decreased testicular testosterone in conjunction with increased serum testosterone, which suggests sec-hormone binding globulin (SHBG) may be affected. In a subsequent study, these researchers dosed rats orally for 15-30 days. Under the conditions of this study, decreases in testicular testosterone, plasma testosterone, LH, and FSH as well as decreased steroidogenic enzyme and cytochrome P-450-dependent monooxygenase were reported. These decreases in LH may lead to decreases in the activity of Steroidogenic Acute Regulatory Protein (responsible for translocation of cholesterol to the inner mitochondria) and may therefore affect the conversion of cholesterol to testosterone. Vonier et al. (1996) conducted a competitive binding assay using alligator oviduct tissue and found endosulfan exposure significantly inhibited 3 H-17-estradiol binding to the estrogen receptor and progestin 3 H-R5020 binding to the progesterone receptor. Ramamoorthy et al. used the yeast reporter system to discover endosulfan induced human-ER-mediated-gal activation. Endosulfan induced galactosidase transcription/expression to about 32% of the induction seen after estradiol treatment at 0.01 µM. In a study conducted by Sinha et al. (1995) rats dosed orally with endosulfan for 70 days exhibited decreases in sperm counts in the cauda epididymis as well as decreased intratesticular spermatid counts. Finally, Lakshmana et al. (1994) showed endosulfan induces small but significant changes in the levels of noradrenaline, dopamine and serotonin in the developing rat brain and deficits in the operant learning performance suggesting possible effects on the neuroendocrine system."*

The examples of indirect endocrine action cited by EPA in this report were addressed in the ETF document submitted in January 2001 (MRID# 45300203). In the ETF document a summary of the available public literature in vivo and ex vivo androgenic assays was provided, giving the endpoints evaluated and the results. In most cases the administrated

dose in these studies was within the systemically toxic range for endosulfan, based on guideline subchronic exposure studies. In the majority of these studies evaluation of potential liver and kidney toxicity was not conducted, nor was any histopathological evaluation conducted on critical sex organs. The absence of this type of data severely limits the ability to assess the relevance of the stated findings. While the Agency has included indirect effects within the scope of defining endocrine disruption, when the effects noted are secondary to frank toxicity of key homeostatic organ systems, the relevance to human risk is highly questionable

1) *In vivo data*

The Agency has relied on a summary of public literature prepared by ATSDR on endosulfan with regard to potential hormonal effects from *in vivo* testing in rats (e.g. serum and testicular testosterone levels, androgen enzyme induction, spermatological endpoints and in vitro binding assays). The citations provided in the FQPA Safety Factor Committee only represents one side of the available data, and not consistent with a science-based weight-of-evidence evaluation. As was summarized by ATSDR, the evidence from in vitro testing is mixed with equal numbers of positive and negative findings. However, as was addressed in Table 1, in vivo testing has not shown any endocrine disruption potential in females, and limited indications of potential disruption of reproductive hormones in males. The weight of this evidence in males must be interpreted with caution, as recent validation efforts in male endocrine assays has shown sperm and hormone parameters to be highly variable, and sensitive to exogenous influences (e.g. circadian fluctuations and stress).[7,8]

With regard to potential effects in male rats, the FQPA Safety Factor Committee cited Singh and Pandey (1989 and 1990) and Sinha *et al.* (1995). While the Committee suggested potential effects on hormonal transport (effects on sec-hormone binding globulin (SHBG) and synthesis (decreased activity of steroidogenic acute regulatory protein (StAR)), there is no evidence in any of these studies, or other available data to support these hypotheses.

An initial evaluation of these studies indicates a variety of technical issues that severely limit the weight-of-evidence they provide toward the overall assessment of potential effects in male rats due to endosulfan exposure. In Singh and Pandey (1989), the serum and testicular testosterone values were not dose-dependent and were highly variable over the two time points (day 7 and day 15). The values were also inconsistent when compared to levels of testicular steroidogenic enzyme levels. Variable decreases and increases in testicular activity levels of 3ß-hydroxysteroid dehydrogenase (responsible for conversion of androstenediol to testosterone) and 17ß-hydroxysteroid dehydrogenase

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[7] **Andrews et. al.** ***Feasibility and potential gains of enhancing the subacute rat study protocol (OECD test guideline no. 407) by additional parameters selected to determine endocrine modulation. A pre-validation study to determine endocrine-mediated effects of the antiandrogenic drug flutamide.*** **Arch Toxicol (2001) 75:65-73.**

[8] **Ulbrich B. and Palmer A.K.,** ***Detection of Effects on Male Reproduction A Literature Survey.*** **J. American Col. Of Toxicol. Vol. 14, pp.293-327. 1995**

(responsible for conversion of androstenedione to testosterone) did not correspond to decreases and increases in testicular testosterone. ATSDR also concluded "*results after a 15-day exposure were highly variable and frequently not dose-related, making interpretation of the significance of the study's results difficult*."[9]

In the subsequent study, Singh and Pandey (1990), animals were dosed at 7.5 and 10 mg/kg (known systemically toxic doses) for 15 or 30 days. Decreases in plasma levels of LH, FSH and testosterone, as well as testicular testosterone levels were detected. Again, the changes in levels were variable, with only the plasma levels being dose-dependent. There was no change in testis weight and no information on histopathology. While the Agency hypothesized that the decrease in Lutenizing Hormone (LH) resulted in decreased testicular testosterone via potential interruption of synthesis through effects on Steroidogenic Acute regulatory Protein (StAR), there is no evidence to support this supposition. However, endosulfan does cause liver enzyme induction, which is known to result in increased steroid metabolism and clearance (Wilson, 1997; Singh SK and Pandey RS, 1989a and 1990). In addition, new investigations using juvenile rat Leydig cells showed no effect of endosulfan on testosterone levels or conversion of 22(R)hydroxycholesterol to testosterone (Murono EP, 2001). Therefore, the decrease in testosterone levels, seen at doses known to cause liver and kidney toxicity (Dikshith et al. 1984; Singh and Pandey 1989b), is more likely a direct result of increased metabolism and excretion of steroid hormones, than a protracted effect on synthesis and transport.

Studies have shown that liver enzyme induction results in rapid metabolism of testosterone to dihydroxytestosterone (DHT), increasing uptake of intracellular testosterone in the testis and increasing the plasma to testis testosterone ratio.[10] This is a transient and fast metabolic shift, which is readily reversible, as was shown in Singh and Pandey (1990) where a complete recovery of hormones was noted 7 days post dosing. Taken together, the results of these studies are highly variable, supporting the recent findings in endocrine assay validation reports that found sperm parameters and hormone levels of minimal predictive value, while histopathology provided the earliest and most accurate prediction of endocrine-mediated effects.[11]

The Agency also cited Sinha *et al.* (1995) in which mature rats were dosed via gavage with endosulfan at levels of 2.5, 5.0 and 10.0 mg/kg/day for 70 days. Decreases in sperm count in the cauda epididymis and spermatid counts were reported. There was no information provided on hormone levels or histopathology of the testis. While there was no apparent change in body weights, the doses used are within the range of known systemically toxic levels. As stated above, in the absence of better information, these effects provide limited evidence of an endocrine-mediated effect. Again, transient

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[9] ATSDR (2000) ***Toxicological Profile for Endosulfan*****. P. 75**

[10] Martin C.R., Endocrine Physiology, ***The mal reproductive system,*** **Oxford University Press, New York, 1978. Chapter 20, p. 252**

[11] Andrews et. al. ***Feasibility and potential gains of enhancing the subacute rat study protocol (OECD test guideline no. 407) by additional parameters selected to determine endocrine modulation. A pre-validation study to determine endocrine-mediated effects of the antiandrogenic drug flutamide. Arch Toxicol (2001) 75:65-73.***

depression of testosterone due to increased metabolic clearance through a full cycle of spermatogenesis (approximately 60 days in rats) would be expected to result in temporary decreases in sperm production.

2) *In vitro data*

The Agency cited in vitro testing of endosulfan regarding the potential of endosulfan to affect estrogenic binding and estrogen-mediated cellular activity (Vonier *et al.*, 1996 and Ramamoorthy *et al.,* 1997). As was reported in previous ETF responses (MRID# 44939102, dated October 4, 1999; and MRID# 45300203, dated January 5, 2001), as well as in the ATSDR summary, there are numerous published studies showing no *in vitro* or *in vivo* estrogenic activity of endosulfan. In contrast to Vonier *et al.* (1996), a second competitive binding assay, showed that neither of the endosulfan isomers, singly or in combination with dieldrin, inhibited 17 β -estradiol binding to recombinant human ER at concentrations up to 10 μM (Arcaro et al. 1998) or to mouse uterine receptor (Shelby et al. 1996). Similarly, 17 β -estradiol-induced foci formation in MCF-7 human breast cancer cells was neither inhibited nor stimulated by co-treatment with endosulfan (Arcaro et al. 1998). In addition, in contrast to findings by Ramamoorthy *et al.,* (1997), endosulfan at 10 μM had no effect on β -gal activity in yeast *(Saccharomyce*s) transfected with either the human or rainbow trout ER (Andersen et al. 1999). There was also no effect observed on transcriptional activation of HeLa cells transfected with plasmids containing an estrogen receptor as a responsive element (Shelby et al. 1996). Endosulfan also did not induce transient reporter gene expression in MCF-7 human breast cancer cells at an incubation concentration of 2.5 μM (Andersen et al., 1999).

Based on an exhaustive summarization of in vitro and in vivo studies, the ATSDR concluded "*endosulfan is neither estrogenic nor disruptive of thyroid or pituitary hormone levels in females <u>in vivo</u>, despite its weak estrogenicity in several <u>in vitro</u> test systems."*p.141

The weight-of-evidence provided by the full spectrum of public literature and guideline studies continue to support the ETF's conclusions that endosulfan is unlikely to be an endocrine disruptor in humans.

Page 3, Section I. Hazard Assessment: 4. *"Neuroendocrine"*

Finally, the FQPA Safety Factor Committee has referenced a study by Lakshmana et al. (1994) that showed a highly variable pattern of monoamine concentrations in various brain regions of rats exposed from PND 1-10 or PND 1-25 via gavage at 6.0 mg/kg/day. The change in levels of monoamines was not consistent over time or region, and the author had no explanation of the changes or how the results maybe related to any neuroendocrine effects.

Table 2: Monoamine activity levels in rat brain (Lakshmana M.K. and Raju T.R/ Toxicology 91,1994, p.139-150)

Monoamine levels following oral administration of endosulfan at 6 mg/kg/day (peanut oil) from PND 2 – 10 or PND 2-25							
Brain	Day 10				Day 25		
Region	Noradrenaline	Dopamine	Serotonin		Noradrenaline	Dopamine	Serotonin
Olfactory bulb	12% inc**	27% dec**	12% inc*		NC	14% inc*	NC
hippocampus	NC	42% dec***	41% inc**		20% inc**	45% dec***	NC
visual cortex	NC	NC	30% inc**		NC	NC	NC
brainstem	10% inc*	NC	15% inc**		NC	NC	20% dec*
cerebellum	NC	NC	NC		12% inc*	NC	31% dec**

*p<0.05; **p<0.001; ***p<0.001

At 25 days of age rats were subjected to an operant learning test (Skinner - food reward). Endosulfan treated animals showed a significant increase in acquisition time (learning) and a decrease in pedal presses (reward – associated with memory). While the animals in this test did not show any effects on body weight, studies by Paul et al. (1993, 1994 & 1995) showed decreased body weight in immature rats at 2 mg/kg for 90 days and increased mortality in adult females at 6 mg/kg in diet for 30 days. Guideline subchronic studies had also shown signs of systemic toxicity at doses as low as 1.5 mg/kg (liver histopathological changes and body weight). Since this was a food stimulated reward test, systemically toxic manifestations such as lack of appetite and lethargy cannot be ruled out. In addition, the decrease or lack of change in serotonin at day 25 is not consistent with other studies, which looked at serotonin levels in the cerebellum and midbrain, and showed some effect of adult and immature animals on conditioned avoidance tests (Paul et al. 1993, 1994 & 1995; Zaidi et al. 1984; and Agrawal et al. 1983). The lack of consistency in monoamine levels and the absence of sensitivity in responses between adults and younger animals limit the value of these studies in a weight-of-evidence evaluation. This is especially true since a request made by HIARC in their October 1998 review that "this study be reviewed/evaluated and that a DER be prepared," has never been acknowledged or acted upon by the Agency. The absence of an internal peer review by the Agency of any of these published sources, is a serious oversight and compromises the scientific assessment and rationale provided by the FQPA Safety Factor Committee.

Conclusions

Based on the data presented, the ETF has concluded that there is no evidence of enhanced susceptibility to younger animals and existing reliable data do not demonstrate a potential for endocrine disruption in males or females. The assessment by the FQPA Safety Factor Committee of a 10x is excessive and not justified. Concerning the overall weight-of-evidence it is prudent to rely on acceptable guideline studies before using the open literature data that might not meet EPA's standard acceptance criteria and are often not reproducible. Therefore, we would appreciate if the Agency would take the time to

review our comments and reconsider the recent 10x FQPA Safety Factor Assignment, which will substantially affect the preliminary dietary risk assessment for endosulfan.

Our conclusion is based on the following:

- Available data from both submitted guideline studies and published sources do not indicate increased sensitivity of young rats.
  - Published sources provided limited evidence of age-related effects due to: 1) inconstancies in findings between laboratories and strains of rats (Dalsenter et al. (1999) vs. (Sinha 2001); 2) lack of statistical significance in sperm morphology endpoints; 3) lack of histopathological evidence of effects on gonadal organs; and 4) use of spermatological and hormonal endpoints which have been shown to be highly variable, sensitive to exogenous influences and poorly predictive of endocrine-mediated effects.
  - Submitted guideline studies, which provide substantive and reliable data concerning potential developmental and reproductive effects, showed no evidence of effects on sexual differentiation, maturation or fertility. There no reported histopathological effects on any gonadal organs (male or female) in either generation.
  - ATSDR concluded "*No reports of adverse effects in endosulfan-exposed children were found, but it is reasonable to assume that children will exhibit similar signs and symptoms to those in adults under similar exposure conditions. Some studies in animals have provided evidence that young animals respond to endosulfan differently than adult animals (Kiran and Varma 1988; Lakshmana and Raju 1994; Sinha et. al. 1995, 1997; Zaidi et. al. 1985), but there is no conclusive evidence to suggest that young animals are more susceptible than older ones."*[12]

- There was no new or additional evidence provided that showed endosulfan to be a potential endocrine disruptor.

  - The submitted guideline studies did not provide any treatment-related evidence of endocrine disruption in males or females. Effects noted in a NCI 1978 chronic toxicity study in rats (testicular atrophy and parathyroid hyperplasia) were secondary to frank toxicity and chronic renal failure. There was no statistically-significant difference in pituitary weights between F_0 female pups and concurrent controls (excluding a single outlying animal), and there is significant *in vivo* evidence to show that the uterine is not a target organ of endosulfan. Therefore, the uterine weight change seen in the F_{1b} female pups (1^{st} mating), in the absence of histopathological effects, are not of toxicological significance and do not provide evidence for endocrine disruption.

  - Weight-of-evidence of all available *in vitro* data concerning endocrine-mediated effects resulting from exposure to endosulfan are inconclusive and not supported by current *in vivo* data.

[12] **ATSDR Toxicity Profile for Endosulfan. September 2000. P.166**

- ATSDR concluded, "*endosulfan is neither estrogenic nor disruptive of thyroid or pituitary hormone levels in females in vivo, despite its weak estrogenicity in several in vitro test systems.*"p.141

- The published sources reporting spermatogenetic effects in rats showed highly variable results that provided limited information on parameters that have been shown to be poorly predictive of endocrine-mediated effects, especially in the absence of histopathology.

- The single reference cited for neuroendocrine effects in young rats showed highly variable levels of monoamines in the brain of young rats, which were not consistent spatially or temporally. The author had no explanation for the effects noted and was not able to provide a scientific rational for the results. In the absence of a full review of this data, the relevance of the results reported unknown and adds little value to the weight-of-evidence evaluation for endosulfan.

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ATTACHMENT A

Endosulfan Reproductive Toxicity Study (MRID 00148264): Pituitary Weights in Females Pups 1st Mating Fo Generation								
Group 1: Control			Group 4: 75 ppm - all females			Group 4: 75 ppm - w/o 245		
Rat No.	Body Wgt. (g)	Pituitary Wgt. (g)	Rat No.	Body Wgt. (g)	Pituitary Wgt. (g)	Rat No.	Body Wgt. (g)	Pituitary Wgt. (g)
129	47.71	0.0029	225	30.73	0.0059	225	30.73	0.0059
130	39.03	0.0016	226	52.95	0.0036	226	52.95	0.0036
131	40.25	0.0044	227	47.73	0.0031	227	47.73	0.0031
132	49.55	0.0043	228	29.3	0.0025	228	29.3	0.0025
133	38.35	0.0035	229	63.34	0.0078	229	63.34	0.0078
134	37.06	0.0042	231	31.19	0.0025	231	31.19	0.0025
135	42.64	0.0052	232			232		
136	53.33	0.0054	233	33.47	0.004	233	33.47	0.004
137	40.52	0.0034	234	39.33	0.0045	234	39.33	0.0045
138	46.51	0.0030	235	59.09	0.0035	235	59.09	0.0035
139	46.99	0.0038	236	38.61	0.0046	236	38.61	0.0046
141	42.77	0.0018	237	35.57	0.0056	237	35.57	0.0056
142	33.05	0.0038	238	48.36	0.0046	238	48.36	0.0046
143	49.15	0.0013	239	61.14	0.0037	239	61.14	0.0037
144	37.65	0.0019	240	42.46	0.0046	240	42.46	0.0046
145	48.12	0.0039	241	50.06	0.0061	241	50.06	0.0061
146	37.30	0.0061	242	37.56	0.0027	242	37.56	0.0027
148	39.91	0.0032	243	51.19	0.0057	243	51.19	0.0057
149	42.86	0.0023	244	31.42	0.0044	244	31.42	0.0044
150	40.04	0.0041	245	51.76	0.0109	245		
151	47.99	0.0015	246	28.36	0.0086	246	28.36	0.0086
152	45.46	0.0059	247	39.96	0.0041	247	39.96	0.0041
153	39.22	0.0034	248	49.96	0.0041	248	49.96	0.0041
154	36.78	0.0027	249	43.52	0.0056	249	43.52	0.0056
155	42.19	0.0041	250	40.12	0.0051	250	40.12	0.0051
156	41.35	0.0018	251	39.44	0.0037	251	39.44	0.0037
157	59.80	0.0052	252	36.44	0.0026	252	36.44	0.0026
[1]158	NR		253	30.18	0.0019	253	30.18	0.0019
159	39.77	0.0075	254			254		
160	58.35	0.0054	255	23.94	0.0043	255	23.94	0.0043
			256	27.54	0.0043	256	27.54	0.0043
Means	43.58	0.0037	Means	41.20	0.0046		40.82	0.0044
		P value						P Value
T-Test with all animals		0.047238529			T-Test without outlier (#245)			0.088505

ATTACHMENT B
Reproductive Toxicity Study
Summary

Title:	Effect of Endosulfan Technical (Code: HOE 02671 OI AT209) on Reproductive Function of Multiple Generations in the Rat
Laboratory :	Report HST 204/83768 (A29428); EPA MRID# 00148264
Experimental work:	From 4/21/1982 to 12/13/1983
Test material :	HOE 02671 OI AT209, purity 97%
Methodology :	MAFF (Japan, Jan1985), EPA FIFRA (Nov 1984)
GLP conformity :	Yes

Material and Methods:

Four groups of 32 male and 32 female Crl: COBS CD® (SD) BR rats received endosulfan technical continuously via the diet at concentrations of 0, 3, 15, and 75 ppm for 10 weeks pre-mating and throughout mating, gestation, and lactation. The F1 animals selected to remain on study as the next generation (28/sex/group) were offered diets at the same concentrations as their parents from weaning for at least 10 weeks before mating, and throughout mating, gestation, and lactation of the F2 litters. Clinical observations, body weights, body weight changes, water and food consumption, reproduction, and litter data were recorded.

According to food consumption throughout the treatment period, group mean achieved dosage were as follows:

Dose (ppm)	**3**	**15**	**75**
Female Dose (mg/kg/day)	0.2	1.2	6.2
Male Dose (mg/kg/day)	0.2	1.0	5.0

Summary of effects:

1. Clinical signs

F0: There were no test material-related clinical observations for F0 adults given 3, 15, or 75 ppm or F1 offspring from any of the treated groups.

F1: There were no test material-related clinical observations for F1 adults given 3, 15, or 75 ppm or F2 offspring from any of the treated groups.

2. Mortality

F0: Single mortalities in females occurred in the control group and at 3 and 15 ppm. There were no mortalities at 75 ppm in either the males or females.

F1: There was single female death in the F1B generation in the control group. There were no mortalities in any of the other dose groups.

3. Bodyweight

At 75 ppm F0 generation females and both F1 males and females showed marginally lower mean weekly weight gains, and during gestation at first mate of both generations in comparison with controls. Among F0 females the difference was statistically significant ($p<0.05$) at week 4 only. There were no other statistically significant differences and F0 males at 75 ppm showed slightly higher weight gain than among control animals.

4. Food consumption

Food consumption in the F1 males at 75 ppm showed slightly lower values throughout the dosing period. No other dose groups were affected.

5. Reproduction Data

F0: There were no effects noted on mating performance, pregnancy rate or gestation periods at any dose.

F1: There were no effects noted on mating performance, pregnancy rate or gestation periods at any dose.

Table 1: Fertility Indices in F0 generation

Dose Level		0 ppm	3 ppm	15 ppm	75 ppm
First Mating					
Number of paired females	N	32	32	32	32
Total number inseminated	N	31	32	29	32
	%	97	100	91	100
Total number pregnant	N	31	29	27	31
	%	100	91	93	97
Fertility index Number pregnant/ N° paired	%	97	91	84	97
Second Mating					
Number of paired females	N	32	32	31	32
Total number inseminated	N	31	31	29	32
	%	97	97	94	100
Total number pregnant	N	31	31	29	32
	%	100	100	100	100
Fertility index Number pregnant/ N° paired	%	97	97	94	100

Table 2: Fertility Indices in F1B generation

Dose Level		0 ppm	3 ppm	15 ppm	75 ppm
First Mating					
Number of paired females	N	28	28	28	28
Total number inseminated	N	27	26	26	27
	%	96	93	93	96
Total number pregnant	N	27	26	25	26
	%	100	100	96	96
Fertility index Number pregnant/ N° paired	%	96	93	89	93
Second Mating					
Number of paired females	N	28	28	28	28
Total number inseminated	N	27	28	27	28
	%	96	100	96	100
Total number pregnant	N	27	28	26	27
	%	100	100	96	96
Fertility index Number pregnant/ N° paired	%	96	100	93	96

6. Litter Data

F0: There were no treatment-related effects on litter loss, litter size, pup mortality, sex ratios or mean pup weights. At 75 ppm during lactation to weaning there was a decrease in mean litter weights during both mates, with occasional statistically significant differences ($p<0.05$). However, there was no corresponding effect on pup weight or litter size.

F1: There were no treatment-related effects on litter loss, litter size, pup mortality, sex ratios or mean litter and pup weights.

7. Organ weights

- Relative, but not absolute, liver weights were increased in both male ($p<0.05$) and female ($p<0.01$) F0 adults at 75 ppm. Relative liver weights were also increased in F1B adult females at 15 ppm ($p<0.01$) and 75 ppm ($p<0.001$).
- Relative, but not absolute, increase in heart weight was seen in F0 males at 15 ppm ($p<0.05$) and 75 ppm ($p<0.01$).
- Relative, but not absolute, increase in kidney weights were in F0 and F1b males at 75 ppm ($p<0.01$)
- Relative, but not absolute, brain weight was increased in F0 females at 75 ppm ($p<0.05$)
- Relative, but not absolute, pituitary weight was increased in F0 females of the 1st mating at 75 ppm ($p<0.05$).
- Relative, but not absolute, uterine weight was increased in the F1B females of the 1st mating at 75 ppm ($p<0.01$).

Table 3: Group Mean Liver Weights (g)

	Male				Female			
Dose (ppm)	0	3	15	75	0	3	15	75
F_0 Adults								
Absolute weight (g)	25.66	26.74	26.50	28.35	14.03	14.08	13.70	14.96
Relative weight[1]	26.18	25.97	27.07	28.03*	13.82	13.93	13.81	15.20**
F_0 Weanlings (1st mating)								
Absolute weight (g)	2.57	2.42	2.42	2.50	2.47	2.22	2.34	2.41
Relative weight[1]	2.45	2.42	2.53	2.51	2.34	2.28	2.40	2.42
F_0 Weanlings (2nd mating)								
Absolute weight (g)	2.80	2.63	2.54	2.71	2.66	2.56	2.51	2.54
Relative weight[1]	2.71	2.58	2.60	2.79	2.57	2.51	2.57	2.61
F_1 Adults								
Absolute weight (g)	25.86	27.18	24.91	26.23	13.12	13.68	14.10	14.82
Relative weight[1]	25.86	26.12	25.30	26.90	13.18	13.50	14.22**	14.82***
F_1 Weanlings (1st mating)								
Absolute weight (g)	1.83	2.18	1.78	1.77	1.60	2.00	1.68	1.68
Relative weight[1]	1.96	1.89	1.80	1.90	1.72	1.75	1.69	1.77
F_1 Weanlings (2nd mating)								
Absolute weight (g)	2.13	2.30	2.08	2.19	2.04	2.30	1.96	2.04
Relative weight	2.16	2.16	2.13	2.26	2.08	2.09	2.07	2.13

[1]values adjusted for body weight as covariate
Significantly different from control, *p<0.05, **p<0.01.

Table 4: Group Mean Pituitary Weights (g)

	Male				Female			
Dose (ppm)	0	3	15	75	0	3	15	75
F_0 Adults								
Absolute weight (g)	0.016	0.017	0.017	0.017	0.020	0.018	0.019	0.019
Relative weight[1]	0.016	0.016	0.018	0.017				
F_0 Weanlings (1st mating)								
Absolute weight (g)	0.004	0.003	0.004	0.003	0.004	0.004	0.003	0.005
Relative weight[1]	0.003	0.003	0.004	0.003	0.004	0.004	0.003	0.005*
F_0 Weanlings (2nd mating)								
Absolute weight (g)	0.003	0.003	0.004	0.003	0.003	0.004	0.003	0.003
Relative weight[1]	0.003	0.003	0.004	0.004				
F_1 Adults								
Absolute weight (g)	0.018	0.017	0.016	0.017	0.018	0.020	0.021	0.017
Relative weight[1]								
F_1 Weanlings (1st mating)								
Absolute weight (g)	0.003	0.003	0.003	0.003	0.003	0.004	0.003	0.003
Relative weight[1]	0.003	0.002	0.003	0.003	0.003	0.003	0.003	0.003
F_1 Weanlings (2nd mating)								
Absolute weight (g)	0.004	0.004	0.004	0.003	0.004	0.004	0.003	0.003
Relative weight								

[1]values adjusted for body weight as covariate
Significantly different from control, *p<0.05, **p<0.01.

Table 5: Group Mean Uterus Weights (g)

	Male				Female			
Dose (ppm)	0	3	15	75	0	3	15	75
F_0 Adults								
Absolute weight (g)					0.633	0.623	0.578	0.591
Relative weight[1]								
F_0 Weanlings (1st mating)								
Absolute weight (g)					0.047	0.051	0.050	0.047
Relative weight[1]					0.045	0.052	0.051	0.048
F_0 Weanlings (2nd mating)								
Absolute weight (g)					0.054	0.056	0.057	0.047
Relative weight[1]					0.052	0.055	0.058	0.048
F_1 Adults								
Absolute weight (g)					0.616	0.632	0.645	0.589
Relative weight[1]								
F_1 Weanlings (1st mating)								
Absolute weight (g)					0.035	0.045	0.039	0.043
Relative weight[1]					0.037	0.041	0.039	0.044**
F_1 Weanlings (2nd mating)								
Absolute weight (g)					0.049	0.053	0.049	0.046
Relative weight					0.050	0.050	0.051	0.047

[1]values adjusted for body weight as covariate
Significantly different from control, *p<0.05, **p<0.01.

Table 6: Group Mean Ovaries Weights (g)

	Male				Female			
Dose (ppm)	0	3	15	75	0	3	15	75
F_0 Adults								
Absolute weight (g)					0.087	0.091	0.094	0.089
Relative weight[1]					0.086	0.090	0.094	0.090
F_0 Weanlings (1st mating)								
Absolute weight (g)					0.020	0.020	0.019	0.021
Relative weight[1]					0.019	0.020	0.019	0.021
F_0 Weanlings (2nd mating)								
Absolute weight (g)					0.021	0.021	0.019	0.020
Relative weight[1]					0.021	0.021	0.019	0.020
F_1 Adults								
Absolute weight (g)					0.082	0.087	0.084	0.086
Relative weight[1]					0.083	0.087	0.084	0.086
F_1 Weanlings (1st mating)								
Absolute weight (g)					0.012	0.016	0.014	0.013
Relative weight[1]					0.013	0.014	0.014	0.014
F_1 Weanlings (2nd mating)								
Absolute weight (g)					0.017	0.018	0.016	0.017
Relative weight					0.017	0.017	0.017	0.017

[1]values adjusted for body weight as covariate
Significantly different from control, *p<0.05, **p<0.01.

Table 7: Group Mean Testes Weights (g)

	Male				Female			
Dose (ppm)	0	3	15	75	0	3	15	75
F_0 Adults								
Absolute weight (g)	4.94	4.92	4.81	4.83				
Relative weight[1]	4.95	4.90	4.83	4.82				
F_0 Weanlings (1st mating)								
Absolute weight (g)	0.284	0.269	0.253	0.269				
Relative weight[1]	0.270	0.269	0.266	0.272				
F_0 Weanlings (2nd mating)								
Absolute weight (g)	0.325	0.308	0.298	0.308				
Relative weight[1]	0.314	0.302	0.306	0.317				
F_1 Adults								
Absolute weight (g)	4.64	4.63	4.78	4.66				
Relative weight[1]	1.73	1.72	1.75	1.73				
F_1 Weanlings (1st mating)								
Absolute weight (g)	0.205	0.246	0.205	0.205				
Relative weight[1]	0.217	0.219	0.208	0.217				
F_1 Weanlings (2nd mating)								
Absolute weight (g)	0.260	0.261	0.242	0.248				
Relative weight	0.262	0.246	0.247	0.256				

[1]values adjusted for body weight as covariate
Significantly different from control, *p<0.05, **p<0.01.

Table 6: Group Mean Brain Weights (g)

	Male				Female			
Dose (ppm)	0	3	15	75	0	3	15	75
F_0 Adults								
Absolute weight (g)	2.061	2.090	2.078	2.085	1.851	1.859	1.855	1.883
Relative weight[1]	2.064	2.085	2.081	2.083	1.845	1.854	1.858	1.890*
F_0 Weanlings (1st mating)								
Absolute weight (g)	1.366	1.371	1.339	1.358	1.348	1.289	1.330	1.307
Relative weight[1]	1.350	1.370	1.354	1.361	1.326	1.300	1.339	1.310
F_0 Weanlings (2nd mating)								
Absolute weight (g)	1.423	1.409	1.419	1.421	1.389	1.377	1.335	1.356
Relative weight[1]	1.412	1.403	1.427	1.431	1.378	1.371	1.342	1.365
F_1 Adults								
Absolute weight (g)	2.104	2.109	2.057	2.086	1.947	1.914	1.919	1.958
Relative weight[1]	2.104	2.098	2.061	2.093	1.948	1.909	1.922	1.958
F_1 Weanlings (1st mating)								
Absolute weight (g)	1.315	1.341	1.328	1.294	1.259	1.285	1.271	1.258
Relative weight[1]	1.335	1.297	1.331	1.314	1.280	1.241	1.273	1.277
F_1 Weanlings (2nd mating)								
Absolute weight (g)	1.395	1.399	1.388	1.382	1.351	1.369	1.332	1.318
Relative weight	1.398	1.382	1.394	1.390	1.355	1.340	1.349	1.328

[1]values adjusted for body weight as covariate
Significantly different from control, *p<0.05, **p<0.01.

9. Macroscopic pathology

F_0 Animals: A slight increased incidence both of animals showing enlarged livers and of animals showing enlarged kidneys was seen in males at 75 ppm.

F_1 Animals: No treatment-related effects were noted in any animals.

10. Microscopic pathology

There was no indication of treatment-related histopathological changes in tissue examined from F1B adults and F2B weanlings.

11. Conclusions

The NOAEL for parental toxicity was 15 ppm (1.2 mg/kg/day), and the parental LOAEL was 75 ppm (6.2 mg/kg/day) based on decreased body weight. The reproductive and developmental NOAEL was 75 ppm (6.2 mg/kg/day), the highest dose tested. A statistically significant increase in pituitary weights in the F_0 females from the first mating at 75 ppm was due to a single animal and was not supported by any histopathological changes. A statistically significant increase in uterine weight in the high dose females of the F1b 1st mating, was not supported by histopathological change, was not seen in any other generation, and was not seen as a target organ in any other study. Therefore these effects were not considered toxicologically significant.